Oxidoreductases Working Together

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Supporting Information

Oxidoreductases Working Together: Concurrent Obtaining of Valuable Derivatives by Employing the PIKAT Method

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1. General

Recombinant histidine-tagged phenylacetone monooxygenase (PAMO), its M446G mutant (M446G) and 4-hydroxyacetophenone monooxygenase (HAPMO) were overexpressed and purified according to previously described methods.[1] The oxidation reactions were performed using the purified enzymes. One unit (U) of Baeyer-Villiger monooxygenase (BVMO) oxidises 1.0 μM of 4-phenylhexan-3-one **6** to 1-phenylpropyl propionate **7** per minute at pH 8.0 and 20ºC in the presence of NADPH (Sigma-Aldrich). Cell-free extract from overexpressed HAPMO on *E. coli* TOP10 has been obtained following a similar procedure as previously described.[2] Terrific Broth (TB), containing 50 μg mL^{-1} ampicillin and 0.02% L-arabinose, was inoculated with 1% of an overnight preculture of recombinant *E. coli* TOP10 overexpressing HAPMO. The culture was incubated at 200 rpm at 28ºC in an orbital shaker for 24 hours. Cells were harvested by centrifugation (6000 rpm for 10 minutes, 4ºC, A614 rotor), washed and resuspended in 10 mL of 50 mM Tris-Cl pH 7.5. A crude extract was prepared by ultrasonication (70% amplitude, 5 min, 2 sec on/off, 4ºC). Cell debris were removed by centrifugation (10000 rpm for 30 min, 4ºC) resulting in the cell-free extract. The latter was stored at -20ºC before use. Protein concentration of cell free extract was determined by Bradford method using bovine serum albumin (BSA) as standard for the calibration curve.[3]

Lactobacillus brevis ADH (LBADH) and *Thermoanaerobacter* sp. ADH (ADH-T) were purchased from Jülich-Codexis. The amount of ADH used in the different assays was calculated according to the activity data given by the supplier (ADH-T 780 U mL^{-1}, LBADH 3850 U mL^{-1}).

Racemic alcohols (±)-**1a**-**g**, ketones **2a**-**e** and **2g**, esters **3a**-**b** and **3e**, and sulfides **4a** and **4c**-**f** as well as other starting compounds, reagents and solvents were of the highest quality grade available, supplied by Sigma-Aldrich-Fluka and Alfa Aesar. Racemic ketone 4-phenylhexan-3-one [(±)-**6**] was prepared according to the literature, starting from 1-phenylbutan-2-one and using the ethyl iodide and NaOH in a biphasic medium (60% yield).[4] Racemic ester (±)-**7** was prepared by acylation of commercial 1-phenylpropanol with propionic anhydride (90% yield). Ketone **2f** was prepared by biocatalysed oxidation of (±)-1,2-octanediol **1f** in the presence of commercially available ADH-'A' from *Rhodococcus ruber* (Biocatalytics Inc.). Sulfide **4b** was obtained by reaction of commercial 2-phenylethanethiol with sodium and iodomethane in methanol (40% yield). Racemic

sulfoxides (±)-**5a-f** were prepared by chemical oxidation of the corresponding sulfides **4a-f** employing hydrogen peroxide and methanol (yields higher than 80%).

All the synthesised compounds exhibited physical and spectral data in agreement with those reported.[5]

Chemical reactions were monitored by analytical TLC, performed on Merck silica gel 60 F_{254} plates and visualised by UV irradiation. Flash chromatography was performed using Merck silica gel 60 (230-400 mesh). IR spectra were recorded on a Perkin-Elmer 1720-X infrared Fourier transform spectrophotometer using KBr pellets. ^{1}H-NMR, ^{13}C-NMR and DEPT spectra were recorded with TMS (tetramethylsilane) as the internal standard with Bruker AC-300-DPX (^{1}H, 300.13 MHz and ^{13}C: 75.4 MHz) spectrometers. The chemical shift values (δ) are given in ppm and the coupling constants (*J*) in Hertz (Hz). ESI^{+} using a HP 1100 chromatograph mass detector or EI^{+} with a Finigan MAT 95 spectrometer was used to record mass spectra (MS). GC analyses were performed on a Hewlett Packard 6890 Series II chromatograph. For all the analyses, the injector and FID temperature were set on 225ºC and 250ºC, respectively. HPLC analyses were developed with a Hewlett Packard 1100 LC liquid chromatograph. High-resolution mass spectra were obtained with a Bruker Microtof-Q-spectrometer.

2. Determination of absolute configurations

Absolute configurations of alcohols **1a-c**,[6] **1d**,[7] **1e**,[8] **1f**,[9] sulfoxides **5a-c**,[5a] **5e**,[5a] **5f**[5d] as well of ketone **6**[5c] and ester **7**[5c] were determined by comparison of retention times on GC with published data.

Absolute configuration of sulfoxide **5d** was established by comparing the retention times on HPLC for this compound with the one obtained in the asymmetric sulfoxidation of prochiral sulfide **4d** employing (+)-diethyl L-tartrate, $Ti(O\text{-}^{i}Pr)_4$ and TBHP.

3. Experimental procedures

*3.1. Asymmetric oxidation of furfuryl methyl sulfide **4d** employing the Kagan methodology.*[10]

$Ti(O\text{-}^{i}Pr)_4$ (2.96 mL, 10 mmol) and (*R*,*R*)-DET [(+)-diethyl L-tartrate] (3.42 mL, 20 mmol) were dissolved at room temperature in 50 mL of CH_2Cl_2 under nitrogen. Distilled water (90 μL, 5 mmol) was then added dropwise. Stirring was maintained until the yellow solution became homogeneous and furfuryl methyl sulfide **4d** was added (640 mg, 5 mmol). The solution was cooled at -20ºC and a TBHP (*tert*-butyl hydroperoxide) solution in decane (1.00 mL, 5.5 mmol) was added. After four hours, additional water was added dropwise (0.9 mL, 50 mmol) to the solution at -20ºC. A strong stirring was maintained for 1 hour at this temperature and for one additional hour at room temperature. The brown gel obtained was filtrated and washed with CH_2Cl_2 (10 mL). The filtrate was kept in the presence of a mixture of NaOH (5%) and brine (30 mL) for 1 h and then extracted with CH_2Cl_2 (3×20 mL). The organic phase was dried over Na_2SO_4 and concentrated to give the crude product, which was purified by flash chromatography using a CH_2Cl_2/MeOH (95/5) mixture as eluent in order to give 96 mg (13% yield) of (*R*)-furfuril methyl sulfoxide.

(*R*)-*Furfuryl methyl sulfoxide*, (*R*)-**5d**. R_f (95/5 CH_2Cl_2/MeOH): 0.30. Mp : Brown oil. IR (KBr): υ 3072, 2923, 1645, 1500 and 1017 cm^{-1}. ^{1}H-NMR ($CDCl_3$-d_1, 300.13 MHz): δ 2.51 (s, 3H), 4.00 (d, 1H, $|^2J_{HH}|$ 12.3 Hz), 4.09 (d, 1H, $|^2J_{HH}|$ 12.3 Hz), 6.38 (s, 2H), 7.41 (s, 1H). ^{13}C-NMR ($CDCl_3$-d_1, 75.4 MHz): δ 37.9 (CH_3), 52.5 (CH_2), 111.1 (CH), 111.2 (CH), 143.5 (CH) and 143.8 (C). MS (ESI^+, *m/z*): 167 [$(M+Na)^+$, 100%]. HRMS (ESI^+) calcd. for $C_6H_8O_2SNa$ $(M+Na)^+$: 167.0137; found: 167.0140. $[\alpha]_D^{25} = -4.3$ (*c* 0.58, $CHCl_3$), *ee*=33%.

*3.2. General procedure for the concurrent biooxidation of secondary alcohols **1a-f** and prochiral sulfides **4a-f** employing ADHs and BVMOs.*

Racemic alcohols (±)-**1a-f** (30 mM) were added to Tris-HCl buffer (50 mM, pH 7.5, 0.5 mL) containing 1% v v^{-1} DMSO. Then, NADPH (0.2 mM), the corresponding ADH (2 U), the BMVO (2 U), and the prochiral sulfide **4a-f** (15 mM) were added. When LBADH was used, magnesium chloride (1 mM) was added to the reaction medium. The mixture was shaken at 30ºC (when PAMO or M446G mutant were used) or 20ºC (when employing HAPMO) and 250 rpm for the times established in Tables 1 and 2. Once finished, reactions were stopped by extraction with ethyl acetate (2 x 0.5 mL) and the organic layer was dried over Na_2SO_4.

Conversions and enantiomeric excesses of final compounds were determined by GC and HPLC analysis.

*3.3. PIKAT of (±)-2-octanol **1a** and benzyl methyl sulfide **4a** employing LBADH and HAPMO cell free extract.*

(±)-2-Octanol [(±)-**1a**] (25 mg, 0.19 mmol) was dissolved in a Tris-HCl buffer (50 mM, pH 7.5, 5.5 mL), containing $NADP^+$ (0.2 mM), $MgCl_2$ (1.0 mM) and DMSO 1% v v^{-1}. To this solution, benzyl methyl sulfide (**4a**) (12 mg, 0.09 mmol) as well as LBADH (30 U) and HAPMO-CFE (500 μL) were added. The reaction was incubated at 20ºC under orbital shaking (250 rpm) for 24 h. Then, the reaction mixture was extracted with Et_2O (5 x 5 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was evaporated at low temperature. The crude reaction was purified by flash chromatography (CH_2Cl_2/Et_2O, mixtures of increasing polarity), in order to obtain (*S*)-**1a** (10.8 mg, 78% yield, 91% *ee*) and (*S*)-**5a** (9.3 mg, 75% yield, ≥99% *ee*). A 9% of hexyl acetate **3a** was achieved.

*3.4. Optimisation of the NADPH concentration when performing the concurrent biooxidation of (±)-**1a** and prochiral sulfide **4a** or racemic ketone (±)-**6**.*

Racemic 2-octanol (±)-**1a** (11.3 or 30 mM) was added to Tris-HCl buffer (50 mM, pH 7.5, 0.5 mL) containing 1% v v^{-1} DMSO and magnesium chloride (1 mM). Then, NADPH (different concentrations), LBADH (2 U), the corresponding BMVO (2 U), and racemic 4-phenylhexan-3-one (±)-**6** (11.3 mM) or sulfide **4a** (15 mM) were added. The mixture was shaken at 30ºC or 20ºC and 250 rpm for the times established. Once finished, reactions were stopped by extraction with ethyl acetate (2 x 0.5 mL) and the organic layer was dried over Na_2SO_4. Conversions and enantiomeric excesses of the resulting compounds were determined by GC and HPLC analysis.

4. GC and HPLC analyses

For the determination of enantiomeric excesses by GC a Restek RT-BetaDEXse (30 m x 0.25 mm x 0.25 µm, 12 psi N_2) column was employed.

Table S1. Determination of *ee* values by chiral GC.

Compound	Program[a]	Retention times [min]
(±)-**1a**[b]	90/5/2.5/105/0/5/130/2/20/180/3	16.3 (*R*), 14.6 (*S*)
(±)-**1b**[b]	90/5/2.5/105/0/5/130/2/20/180/3	23.1 (*R*), 22.5 (*S*)
(±)-**1c**[b]	90/5/2.5/105/0/5/130/2/20/180/3	16.2 (*R*), 14.4 (*S*)
(±)-**1d**[b]	90/5/2.5/105/0/5/130/2/20/180/3	11.7 (*R*), 10.6 (*S*)
(±)-**1e**[b]	90/5/2.5/105/0/5/130/2/20/180/3	10.6 (*R*), 12.2 (*S*)
(±)-**1f**[c]	90/30/20/180/0	26.0 (*R*), 27.2 (*S*)
(±)-**6**	70/5/1/110/0/3/150/0	48.3 (*R*), 49.2 (*S*)
(±)-**7**	70/5/1/110/0/3/150/0	51.5 (*R*), 50.2 (*S*)

[a] Program: initial T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min). [b] Alcohols were derivatised into the corresponding acetate derivatives. [c] Measured as acetonide derivative.

The following column was used for the determination of conversions: Hewlett Packard HP1 (30 m x 0.32 mm x 0.25 µm, 12.2 psi N_2).

Table S2. Determination of conversion values by GC.

Compound	Program	Retention time [min]
1a	70/4/20/200/3	3.3
2a	70/4/20/200/3	3.0
3a	70/4/20/200/3	3.5
1b	70/4/20/100/0/5/170/0/20/200/0	14.9
2b	70/4/20/100/0/5/170/0/20/200/0	13.0
3b	70/4/20/100/0/5/170/0/20/200/0	13.2
1c	70/4/20/200/3	3.3
2c	70/4/20/200/3	3.1
1d	70/4/20/200/3	3.4
2d	70/4/20/200/3	3.1
1e	50/5/2/60/0/20/200/0	6.4
2e	50/5/2/60/0/20/200/0	6.0
3e	50/5/2/60/0/20/200/0	7.2
1f	70/4/20/200/3	7.2
2f	70/4/20/200/3	6.1
1g	70/5/1/110/0/3/150/0/10/200/0	61.4
2g	70/5/1/110/0/3/150/0/10/200/0	62.5
4a	70/4/20/200/3	5.9
5a	70/4/20/200/3	8.7
4b	70/4/20/200/3	15.6
5b	70/4/20/200/3	27.8
4c	70/4/20/200/3	7.9
5c	70/4/20/200/3	9.9
4d	70/5/3/200/0	2.1
5d	70/5/3/200/0	4.8
4e	70/4/20/200/3	4.6
5e	70/4/20/200/3	8.3
4f	70/4/20/200/3	4.8
5f	70/4/20/200/3	7.6

[a] Program: initial T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min)/ slope (ºC min^{-1})/ T (ºC)/ time (min).

For the determination of the enantiomeric excesses of sulfoxides **5a-f**, the following HPLC columns were employed: column A: Chiralcel OD (0.46 cm x 25 cm), column B: Chiralcel OB-H (0.46 cm x 25 cm), both from Daicel.

Table S2. Determination of enantiomeric excesses by HPLC.

Compound	Column	Flow rate [mL min^{-1}]	T [ºC]	Eluent[a]	Retention time [min]
5a	A	0.8	25	*n*-hexane-IPA 9:1	16.8 (*R*); 18.1 (*S*)
5b	B	0.8	25	*n*-hexane-IPA 95:5	36.3 (*S*); 41.4 (*R*)
5c	A	1.0	25	*n*-hexane-IPA 9:1	16.8 (*R*); 18.1 (*S*)
5d	B	0.8	25	*n*-hexane-IPA 9:1	20.7 (*S*); 22.1 (*R*)
5e	B	0.7	25	*n*-hexane-IPA 8:2	7.5 (*S*); 9.0 (*R*)
5f	A	1.0	20	*n*-hexane-IPA 9:1	11.2 (*R*); 14.2 (*S*)

[a] All the experiments were performed with isocratic eluent.

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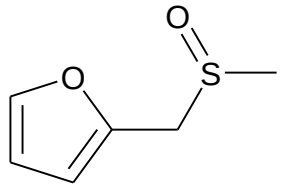
6. Copy of ^{1}H-NMR, ^{13}C-NMR, and DEPT spectra of 5d



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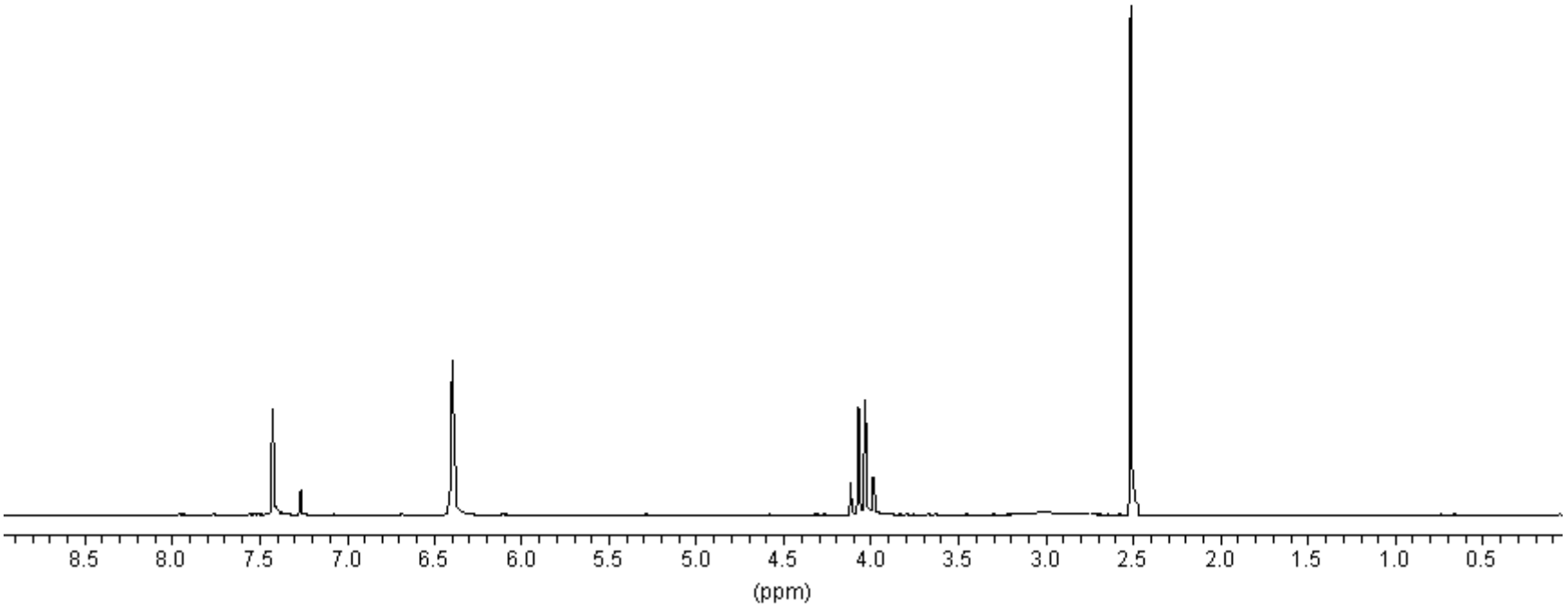

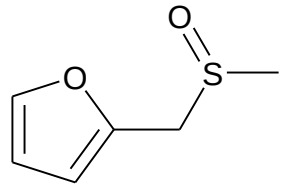
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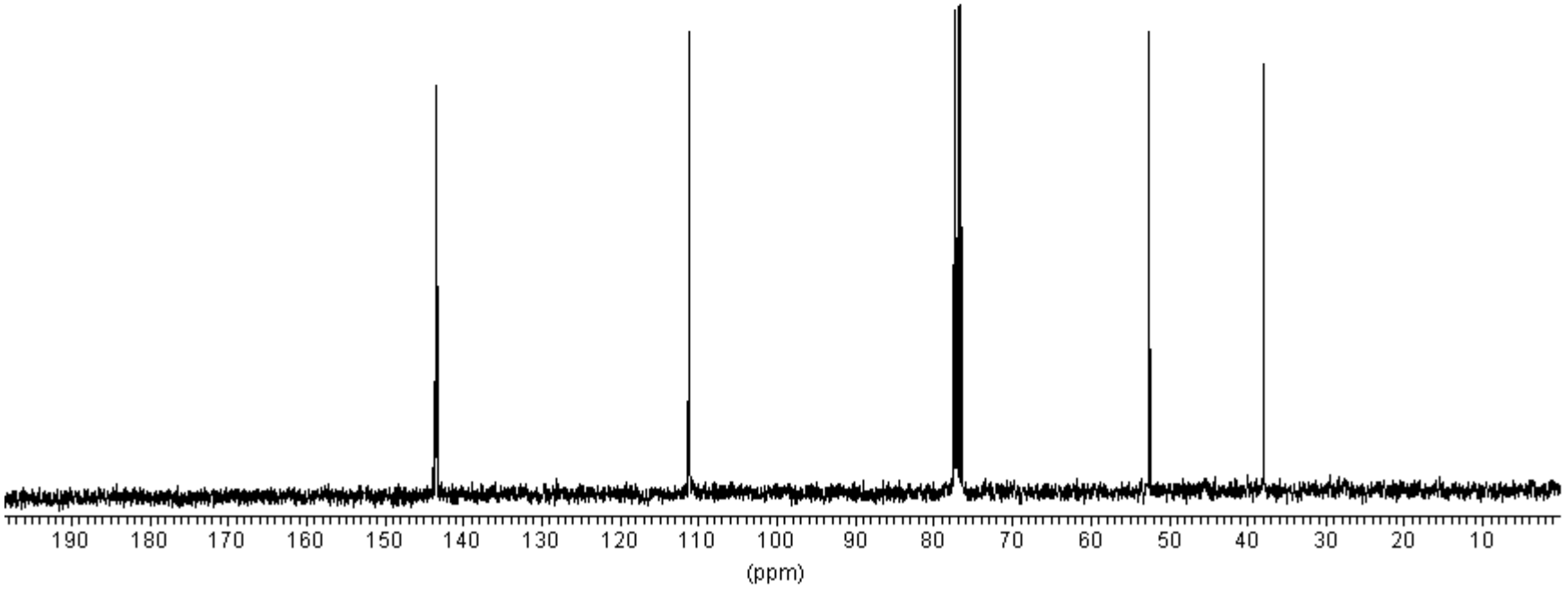
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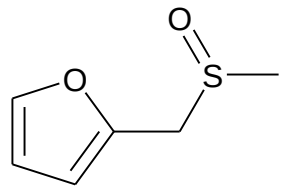

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